

Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1-83. (Cancelled)

84. (New) A method of identifying whether a candidate compound is a modulator of a RUP40 GPCR, wherein the receptor couples to a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 1-1,346 of SEQ ID NO:2;
- (b) amino acids 1-990 of SEQ ID NO:2;
- (c) amino acids 991-1,346 of SEQ ID NO:2;
- (d) amino acids 954-997 of SEQ ID NO:2;
- (e) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;
- (f) amino acids 1-1,349 of SEQ ID NO:4;
- (g) amino acids 1-993 of SEQ ID NO:4;
- (h) amino acids 994-1,349 of SEQ ID NO:4;
- (i) amino acids 954-1000 of SEQ ID NO:4; and
- (j) amino acids 1-141 of SEQ ID NO:6;
or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or 4 or a biologically active fragment thereof;
comprising the steps of:
 - (i) contacting the candidate compound with the receptor; and
 - (ii) determining whether the receptor functionality is modulated;

wherein a change in receptor functionality is indicative of the candidate compound being a modulator of the RUP40 GPCR.

85. (New) The method of claim 84, wherein the candidate compounds are screened as pharmaceutical agents for a heart disease.

86. (New) The method of claim 85, wherein the heart disease is hypertrophic cardiomyopathy or congestive heart failure.

87. (New) A method of identifying whether a candidate compound is a modulator of cardiomyocyte hypertrophy, comprising the steps of:

- (a) contacting the candidate compound with a RUP40 GPCR, wherein the receptor couples to a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:
 - (i) amino acids 1-1,346 of SEQ ID NO:2;
 - (ii) amino acids 1-990 of SEQ ID NO:2;
 - (iii) amino acids 991-1,346 of SEQ ID NO:2;
 - (iv) amino acids 954-997 of SEQ ID NO:2;
 - (v) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;
 - (vi) amino acids 1-1,349 of SEQ ID NO:4;
 - (vii) amino acids 1-993 of SEQ ID NO:4;
 - (viii) amino acids 994-1,349 of SEQ ID NO:4;
 - (ix) amino acids 954-1000 of SEQ ID NO:4; and
 - (x) amino acids 1-141 of SEQ ID NO:6;
- or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the

amino acid sequence of SEQ ID NO:2 or 4 or a biologically active fragment thereof; and

- (b) determining whether the receptor functionality is modulated;

wherein a change in receptor functionality is indicative of the candidate compound being a modulator of cardiomyocyte hypertrophy.

88. (New) A method of identifying whether a candidate compound is a modulator of a cardiovascular disorder, comprising the steps of:

- (a) contacting the candidate compound with a RUP40 GPCR, wherein the receptor couples to a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:

- (i) amino acids 1-1,346 of SEQ ID NO:2;
 - (ii) amino acids 1-990 of SEQ ID NO:2;
 - (iii) amino acids 991-1,346 of SEQ ID NO:2;
 - (iv) amino acids 954-997 of SEQ ID NO:2;
 - (v) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;
 - (vi) amino acids 1-1,349 of SEQ ID NO:4;
 - (vii) amino acids 1-993 of SEQ ID NO:4;
 - (viii) amino acids 994-1,349 of SEQ ID NO:4;
 - (ix) amino acids 954-1000 of SEQ ID NO:4; and
 - (x) amino acids 1-141 of SEQ ID NO:6;
- or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or 4 or said biologically active fragment thereof; and
- (b) determining whether the receptor functionality is modulated;

wherein a change in receptor functionality is indicative of the candidate compound being a modulator of the cardiovascular disorder.

89. (New) The method of claim 88, wherein the cardiovascular disorder is a heart disease.

90. (New) The method of claim 89, wherein the heart disease is hypertrophic cardiomyopathy or congestive heart failure.

91. (New) The method of any one of claims 84, 87 and 88, wherein the RUP40 GPCR is recombinant.

92. (New) The method of any one of claims 84, 87 and 88 wherein said determining is through the measurement of the level of a second messenger selected from the group consisting of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol 1,4,5-triphosphate (IP₃), diacylglycerol (DAG), MAP kinase activity, MAPK/ERK kinase kinase-1 (MEKK1) activity, and Ca²⁺.

93. (New) The method of claim 92, wherein the level of IP₃ or Ca²⁺ is reduced.

94. (New) The method of any one of claims 84, 87 and 88 wherein said determining is through the use of a Melanophore assay, or through the measurement of GTP γ S binding to a membrane comprising said GPCR.

95. (New) The method of any one of claims 84, 87 and 88, wherein the modulator is an agonist, partial agonist, inverse agonist or antagonist of the RUP40 GPCR.

96. (New) The method of any one of claims 84, 87 and 88, wherein the modulator is an inverse agonist or antagonist of the RUP40 GPCR.

97. (New) The method of any one of claims 84, 87 and 88, further comprising the step of comparing the modulation of the receptor caused by the candidate compound to a second modulation of the receptor caused by contacting the receptor with a known modulator of the receptor.
98. (New) The method of any one of claims 84, 87 and 88, further comprising the step of providing the name or structure of the compound or of producing or synthesizing the compound.
99. (New) The method of any one of claims 84, 87 and 88, further comprising the step of formulating the compound into a pharmaceutical composition.
100. (New) A modulator identified according to the method of any one of claims 84, 87 and 88.
101. (New) The modulator of claim 100, wherein the modulator is an agonist, partial agonist, inverse agonist or antagonist of the RUP40 GPCR.
102. (New) The modulator of claim 100, wherein the modulator is an inverse agonist or antagonist of the RUP40 GPCR.
103. (New) A method of modulating the activity of a RUP40 GPCR, wherein the receptor couples to a G protein, said receptor comprising an amino acid sequence selected from the group consisting of:
- (a) amino acids 1-1,346 of SEQ ID NO:2;
 - (b) amino acids 1-990 of SEQ ID NO:2;
 - (c) amino acids 991-1,346 of SEQ ID NO:2;
 - (d) amino acids 954-997 of SEQ ID NO:2;
 - (e) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase chain reaction (PCR) on a human cDNA sample using a specific primer

having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;

- (f) amino acids 1-1,349 of SEQ ID NO:4;
- (g) amino acids 1-993 of SEQ ID NO:4;
- (h) amino acids 994-1,349 of SEQ ID NO:4;
- (i) amino acids 954-1000 of SEQ ID NO:4; and
- (j) amino acids 1-141 of SEQ ID NO:6;

or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or 4 or said biologically active fragment thereof; comprising the step of contacting the receptor with the modulator of claim 100.

104. (New) A method of preparing a composition comprising admixing a modulator identifiable by the method of any one of claims 84, 87 and 88 and a carrier.

105. (New) The method of claim 104, wherein the modulator is an inverse agonist or antagonist of the RUP40 GPCR.

106. (New) A pharmaceutical composition comprising a modulator identified according to any one of claims 84, 87 and 88 and a pharmaceutically acceptable carrier.

107. (New) The pharmaceutical composition of claim 106, wherein the modulator is an inverse agonist or antagonist of the RUP40 GPCR.

108. (New) A method of blocking or decreasing cardiomyocyte hypertrophy comprising providing or administering to a mammal in need of said blocking or decreasing a therapeutically effective amount of a modulator of the mammalian RUP40 GPCR or a pharmaceutical composition comprising the modulator and a pharmaceutically acceptable carrier.

109. (New) A method of preventing or treating a cardiovascular disorder comprising providing or administering to a mammal in need of said preventing or treating a therapeutically effective amount of a modulator of the mammalian RUP40 GPCR or a pharmaceutical composition comprising the modulator and a pharmaceutically acceptable carrier.

110. (New) The method of claim 109, wherein the cardiovascular disorder is a heart disease.

111. (New) The method of claim 110, wherein the heart disease is hypertrophic cardiomyopathy or congestive heart failure.

112. (New) The method of claim 111, wherein the hypertrophic cardiomyopathy results from a disorder selected from the group consisting of:

- (a) post-myocardial infarction remodeling;
- (b) cardiac valve disease;
- (c) sustained cardiac afterload;
- (d) myocarditis; and
- (e) familial hypertrophic cardiomyopathy.

113. (New) The use of claim 108 or claim 109, wherein the modulator is an inverse agonist or antagonist.

114. (New) The method according to claim 108 or claim 109, wherein the mammal is a human.

115. (New) A method of identifying whether a candidate compound is a ligand of a RUP40 GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 1-1,346 of SEQ ID NO:2;
- (b) amino acids 1-990 of SEQ ID NO:2;

- (c) amino acids 991-1,346 of SEQ ID NO:2;
- (d) amino acids 954-997 of SEQ ID NO:2;
- (e) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;
- (f) amino acids 1-1,349 of SEQ ID NO:4;
- (g) amino acids 1-993 of SEQ ID NO:4;
- (h) amino acids 994-1,349 of SEQ ID NO:4;
- (i) amino acids 954-1000 of SEQ ID NO:4; and
- (j) amino acids 1-141 of SEQ ID NO:6;
or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or 4 or said biologically active fragment thereof;
comprising the steps of:
 - (i) contacting the receptor with a labeled known ligand of the GPCR in the presence or absence of the candidate compound; and
 - (ii) determining whether the binding of said labeled known ligand to the receptor is inhibited in the presence of the candidate compound;wherein said inhibition is indicative of the candidate compound being a ligand of the RUP40 GPCR.

116. (New) A method for detecting ligands that bind to a RUP40 GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:
- (a) amino acids 1-1,346 of SEQ ID NO:2;
 - (b) amino acids 1-990 of SEQ ID NO:2;
 - (c) amino acids 990-1,346 of SEQ ID NO:2;
 - (d) amino acids 954-997 of SEQ ID NO:2;
 - (e) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by polymerase

chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8;

- (f) amino acids 1-1,349 of SEQ ID NO:4;
- (g) amino acids 1-993 of SEQ ID NO:4;
- (h) amino acids 994-1,349 of SEQ ID NO:4;
- (i) amino acids 954-1000 of SEQ ID NO:4;
- (j) amino acids 1-141 of SEQ ID NO:6; and
- (k) the amino acid sequence of a G protein-coupled receptor encoded by a polynucleotide hybridizing at high stringency to the complement of SEQ ID NO:1;

or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2 or 4; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or 4 or a biologically active fragment thereof;

comprising the steps of:

- (i) contacting a candidate compound with a host cell or with membrane of a host cell that expresses said receptor, under conditions which permit interaction between said receptor and said candidate compound; and
- (ii) detecting a ligand bound to said receptor.

117. (New) The method of claim 115 or claim 116, wherein the RUP40 GPCR is recombinant.

118. (New) The method of claim 115 or claim 116, wherein the candidate compounds are screened as compounds useful in radio-imaging for identifying a mammal at risk for or progressing toward a cardiovascular disorder.

119. (New) The method of claim 118, wherein the cardiovascular disorder is a heart disease.

120. (New) The method of claim 119, wherein the heart disease is hypertrophic cardiomyopathy or congestive heart failure.

121. (New) The method of claim 118, wherein the mammal is a human.

122. (New) A method for identifying whether a candidate compound is an agent that reduces expression of a mammalian RUP40 GPCR expressed endogenously in a cell or an agent that decreases or blocks a heart disease, said method comprising the steps of:

- (a) contacting or not contacting a plurality of said cells with the candidate compound;
- (b) measuring a level of expression of the mammalian RUP40 receptor in the cells contacted with the candidate compound and a level of expression of the mammalian RUP40 receptor in the cells not contacted with the candidate compound; and
- (c) comparing the level of expression of the mammalian RUP40 GPCR receptor in the cells contacted with the candidate compound with the level of expression of the mammalian RUP40 receptor in the cells not contacted with the candidate compound;

wherein a reduction in the level of expression of the mammalian RUP40 receptor in the cells contacted with the candidate compound compared with the level of expression of the mammalian RUP40 receptor in the cells not contacted with the candidate compound is indicative of the candidate compound being an agent that reduces expression of the mammalian RUP40 GPCR expressed endogenously in the cell or an agent that decreases or blocks a heart disease.

123. (New) The method of claim 122, wherein the cell is a cardiomyocyte.

124. (New) The method of claim 122, wherein the heart disease is hypertrophic cardiomyopathy or congestive heart failure.

125. (New) The method of claim 122, wherein the mammalian RUP40 GPCR is a human RUP40 GPCR.

126. (New) A transgenic non-human mammal comprising expression of a human RUP40 GPCR, said receptor comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 1-1,346 of SEQ ID NO:2;
- (b) amino acids 1-990 of SEQ ID NO:2;
- (c) amino acids 991-1,346 of SEQ ID NO:2;
- (d) amino acids 954-997 of SEQ ID NO:2; and
- (e) the amino acid sequence of an endogenous RUP40 G protein-coupled receptor encoded by a polynucleotide that is amplifiable by performing polymerase chain reaction (PCR) on a human cDNA sample using a specific primer having the nucleotide sequence set forth in SEQ ID NO:7 and a specific primer having the nucleotide sequence set forth in SEQ ID NO:8; or a variant thereof; or a biologically active fragment of the amino acid sequence of SEQ ID NO:2; or a constitutively activated mutant of the amino acid sequence of SEQ ID NO:2 or a biologically active fragment thereof.

127. (New) The transgenic non-human mammal of claim 126, wherein said transgenic non-human mammal exhibits predisposition to or manifest congestive heart failure or hypertrophic cardiomyopathy relative to wild-type control mammal.

128. (New) A method of using the transgenic non-human mammal of claim 126 to identify whether a compound has efficacy for the prevention of or treatment for congestive heart failure or hypertrophic cardiomyopathy, comprising the steps of:

- (a) administering or not administering the compound to the transgenic non-human mammal;
- (b) determining whether administration of the compound has an effect selected from the group consisting of:
 - (i) reduction of wet or dry heart weight;

- (ii) reduction of the wet or dry heart weight/body weight ratio;
- (iii) reduction of the cross-sectional area of myocytes; and
- (iv) reduction of the level of induction of atrial natriuretic factor (ANF) gene;

wherein said determination is indicative of the compound having efficacy for the prevention of or treatment for congestive heart failure or hypertrophic cardiomyopathy.

129. (New) A transgenic non-human mammal comprising a disruption in a RUP40 GPCR gene.

130. (New) The transgenic non-human mammal of claim 129, wherein said transgenic non-human mammal manifests reduced hypertrophic cardiomyopathy on tranverse aortic constriction (TAC) relative to wild-type control mammal.

131. (New) The transgenic non-human mammal of claim 129, wherein said non-human mammal is a mouse and said RUP40 GPCR gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:6.

132. (New) The transgenic non-human mammal of claim 129, wherein said non-human mammal is a rat and said RUP40 GPCR gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:4.

133. (New) An isolated polynucleotide encoding a mouse RUP40 GPCR polypeptide comprising the amino acid sequence of SEQ ID NO:6, or the complement thereof.

134. (New) The isolated polynucleotide of claim 133, wherein the polynucleotide comprises the nucleotide sequence of SEQ IN NO:5.

135. (New) An isolated or recombinant mouse RUP40 GPCR polypeptide comprising the amino acid sequence of SEQ ID NO:6.